

AWARD NUMBER: W81XWH-20-1-0544

TITLE: Designer Nanoparticles" for Immunotherapeutic Treatment of Bladder Cancer

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**CONTRACTING ORGANIZATION: UNIVERSITY OF COLORADO
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**PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012**

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14. ABSTRACT Bladder cancer is a common cancer with more than 70,000 new cases and 15,000 deaths annually in the United States. Bladder cancer is the 4th most common cancer in veterans at 3.6%, behind only prostate, lung and colon cancer. For metastatic, advanced stage bladder cancer, 5 immune checkpoint inhibitors have been approved in the last several years. These drugs have revolutionized the treatment of metastatic bladder cancer, providing durable, and well tolerated responses in ~20% of patients. However, the treatment of localized disease remains unchanged and immune checkpoint inhibitors have not been integrated into the care of these patients. Objective/hypothesis: In this application, we propose to develop a "designer nanoparticle" based on bacteriophage lambda where we have coopted the non-infectious capsid which can be decorated with targeting antibodies, bioactive proteins, DNA, fluorophores, etc., in defined ratios. Specifically, we propose to develop theranostic 'phage-like particles' or PLPs which are decorated with fluorescein, anti-epidermal growth factor receptor (EGFR) and/or CD47 targeting capabilities, and interferon stimulatory DNAs (ISDs) that activate the stimulator of interferon genes (STING) pathway to promote an anti-tumor immune response. We hypothesize that PLPs can be optimized to activate the STING pathway resulting in production of Type I interferons (IFN alpha & beta) leading to a robust and durable anti-tumor immune response. We have generated preliminary data demonstrating engineering of the PLPs and specific uptake into bladder cancer cells. Impact: There is a substantial clinical gap in the treatment of early-stage bladder.					
15. SUBJECT TERMS None listed.					
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1. INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Bladder cancer is a common cancer with more than 70,000 new cases and 15,000 deaths each year in the United States. It is common in both in active and retired military personnel, estimated to be the 4th most common cancer in the Veterans Affairs (VA) medical system. To address this, we will develop a novel immunotherapeutic approach using a “designer nanoparticle” platform that is based upon bacteriophage lambda, a virus that infects bacteria but that is non-infectious in humans. We have coopted the λ capsid which is an icosahedral shaped particle in the nanometer size range that we can then decorate with targeting antibodies, small molecules (e.g. drugs), DNA, bioactive proteins, etc., in a user-defined manner. We propose to develop this designer nanoparticle, a.k.a. ‘phage-like particle’ or PLP, for targeting bladder cancer and activating an anti-tumor immune pathway for treatment of this disease. We are specifically targeting a pathway which was recently recognized to contribute to immune responses against cancer cells. This pathway, the stimulator of interferon genes (STING), when activated, produces anti-tumor proteins termed Type I interferons that promote a robust and durable anti-tumor response by recruitment of other immune cells to attack the tumor.

2. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Bladder cancer, bacteriophage, interferon, STING, immunotherapy

3. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

The major goals for year 1 of the project are:

Goals for year 1	Months	Completion date	Percent completed
1. Production of phage-like particles	1-3	8/30/2021	100%
2. Decoration of phage-like particles (antibodies, ISDs)	1-3	8/30/2021	100%
3. Physicochemical characterization of phage-like particles (SEC, AUC, DLS, EM)	2-4	N/A	100%
4. Assessment of phage-like particle uptake in MB49 and HTB9 cells	4-6	10/15/2021	100%
5. Determine the optimal conditions for activation of the STING pathway in bladder cancer cells (MB49 and HTB9) <ul style="list-style-type: none"> - Measure interferon responses - Measure phosphorylated IRF3 and TBK1 	6-18	N/A	50%

- Measure NF-κB levels and nuclear translocation			
6. Modifications to PLP synthesis based on uptake and activation studies (e.g. decorations, density of antibodies and/or ISDs, etc)	6-18	N/A	0%

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Activities:

During this reporting period, we completed production and decoration of the phage like particles (PLPs). We have completed the majority of characterization of the PLPs and assessed cellular uptake in MB49 bladder cancer cells.

Specific Objectives:

The objectives for this reporting period were to: 1) synthesize, decorate and characterize the PLPs; 2) optimize cellular uptake through modifications of decoration proteins; 3) assess STING pathway activation in bladder cancer cells.

Significant Results: The first significant result was to change our conjugation of the PLPs to include D-cGAMP (a small molecule agonist of the STING receptor) (see new methods for cGAMP conjugation below). This replaced our original interferon stimulatory DNA (ISD) that we previously reported. As shown in Figure 1, we developed a new protocol which significantly improved conjugation of D-cGAMP as shown in the figure on the right of Figure 1 where observe few if any additional bands for D-cGAMP.

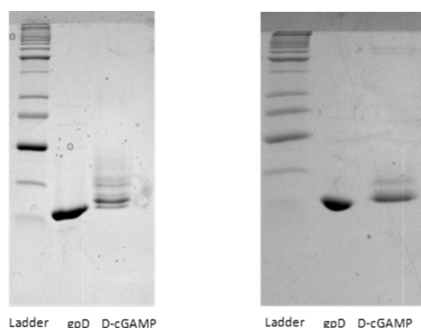


Fig 1. Conjugation and purification of gpD-2'3' cGAMP. (Left) 15% SDS PAGE gel depicting conjugation and purification of the D-cGAMP construct with the old protocol described below. (Right) 15% SDS PAGE gel depicting conjugation and purification of the D-cGAMP construct with the new protocol described above

Secondly, we completed our uptake studies in MB-49 bladder cancer cells. As shown in Figure 2, we observed the greatest amount of cellular uptake of the PLPs when conjugated with 5-10% cetuximab. Going forward we are using those decoration concentrations to examine cell activation. In addition, we have also started studies examining uptake of PLPs in macrophages as these cells would also be present in bladder and could take up the nanoparticles leading to STING activation as a secondary target (data not shown). As shown in Figure 3, we quantitated the uptake data from the confocal imaging. The quantitative data clearly shows that decoration with 5-10% cetuximab is optimal for cell uptake.

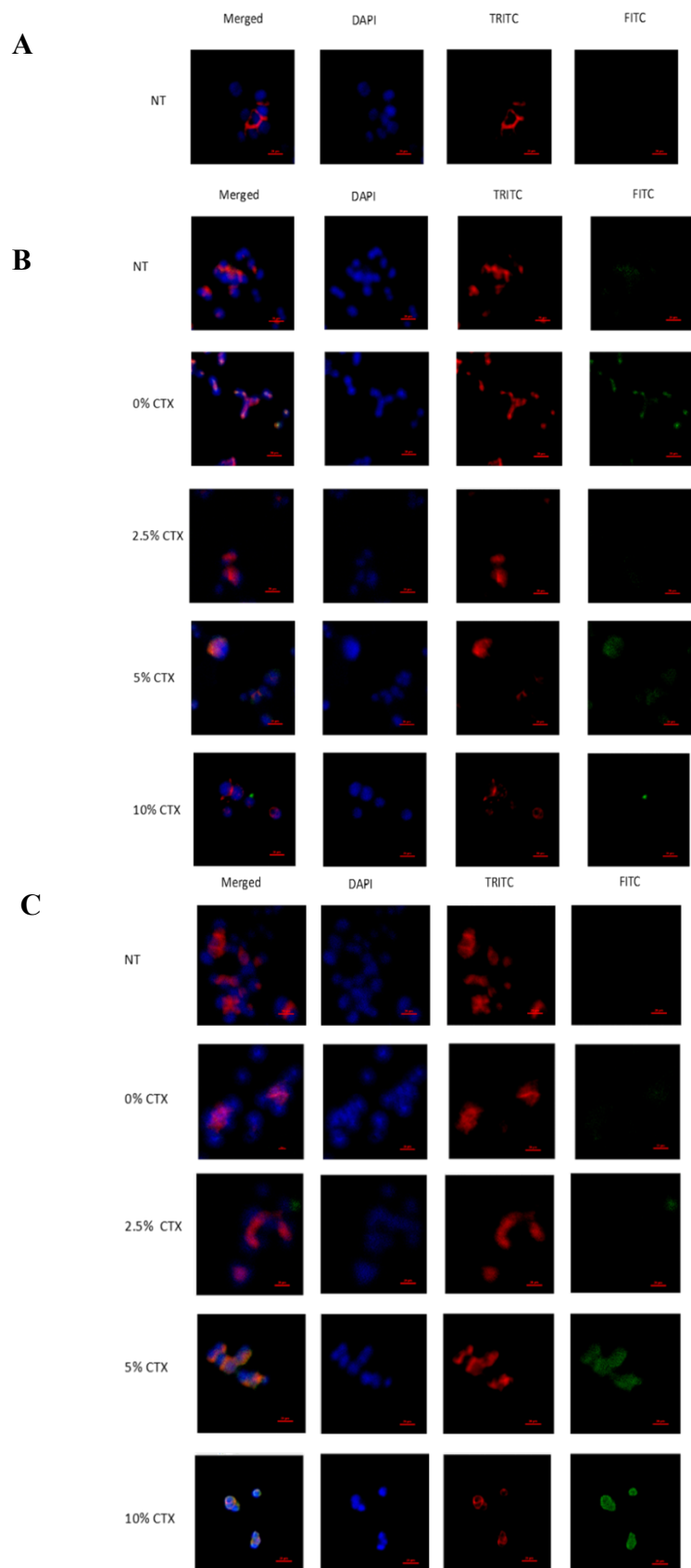


Fig 2. Representative Confocal Microscopy Images: Confocal microscopy images of MB-49 cells using a 9x zoomed view on a 10X air objective. Images are shown representing the merged view and the DAPI, TRITC, and FITC channel individually. (A) MB-49 cells at time point 0 with no treatment. Representative images of MB-49 cells treated with the different Cetuximab percentage PLP's are shown (B) fixed at 30 minutes after treatment administration and (C) fixed at 3 hours after treatment administration.

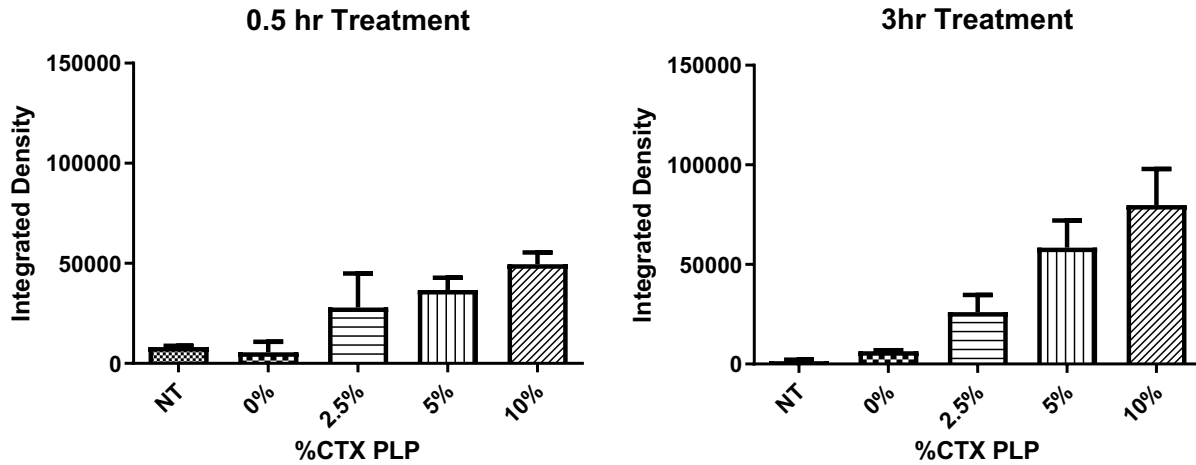


Fig 3. MB-49 uptake of lambda phage like particles with varying ratios of Cetuximab. Graphs charting the integrated density of the FITC channel fluorescence using imageJ (A) 0.5-hour treatment time and (B) 3 hour treatment time. Error bars represent standard error of the mean.

Lastly, we have begun a series of cell activation studies to examine whether the PLPs are able to activate the STING pathway and induce IFN- β production. As shown in Figure 4, we measure IFN- β release from RAW macrophages or MB-49 bladder cancer cells following treatment with D-cGAMP decorated PLPs. The macrophages had a strong IFN- β response with 2nm of PLPs and 50% D-cGAMP decoration (Figure 4A). However, we did not observe IFN- β production in MB-49 bladder cancer cells as shown in Figure 4B. Interestingly, cGAMP by itself did induce IFN- β production. We are not clear why the PLPs appear to work in the macrophages but not in the bladder cancer cells. We are currently troubleshooting this and also examining additional bladder cancer lines.

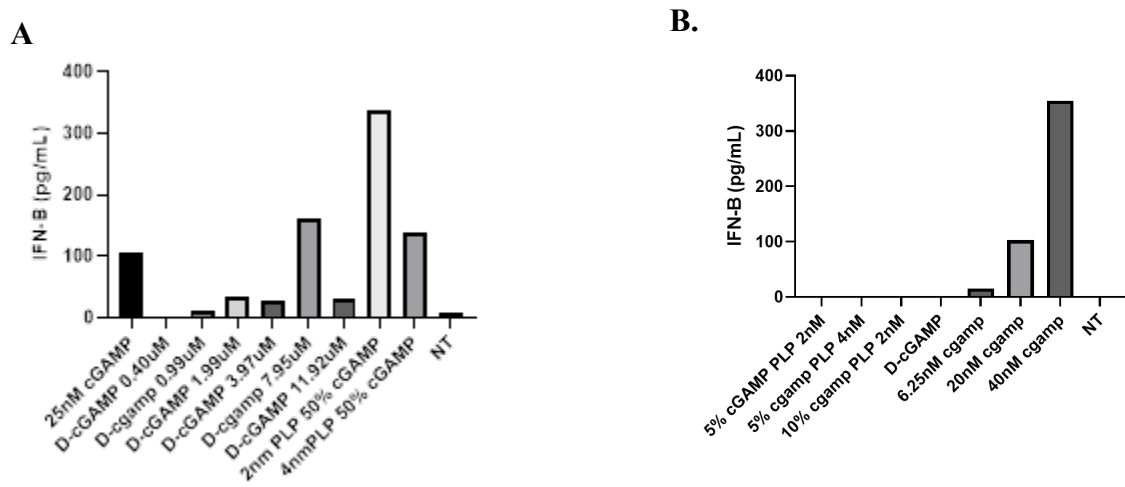


Fig 4. Activation of murine cells gpD-2' 3' cGAMP and 5% cetuximab 50% 2' 3' cGAMP phage like particles. IFN-B response measured by ELISA (A) RAW macrophage cells (B) MB-49 mouse bladder carcinoma cells. n=1 for both graphics.

1. Methods

1. *Cell Culture*: We cultured MB-49 Cells in media with complete DMEM (high glucose with sodium pyruvate), 10% FBS, 10mL of 10X Pen-Strep. We started cultures with 1 million cells from liquid nitrogen. These are quickly thawed and then carefully added to a 15mL tube with 9mL of media. Cells are then spun at 300g for 3 minutes then resuspended and plated in a T-75 flask with 15mL of fresh media. To subculture these cells we collected and spun down the media in the flask at 300g for 5 minutes while trypsinizing the cells in the flask with 5mL of .25X trypsin EDTA for 5 min at 37C. After trypsinization 5mL of fresh media is added to the flask and then all the media is collected in a 15mL tube and spun down at 300g for 5 min. Media from the spins is aspirated from the tube without disturbing the pellet. The cells are then resuspended in fresh media and combined before counting. Cells are then counted by acridine orange staining and 150,000 or 300,000 cells are passed into 15 or 25mL of media depending on if using a T-75 or T-175 respectively. We cultured RAW Cells in media with complete DMEM (high glucose with sodium pyruvate), 10% FBS, 10mL of 10X Pen-Strep. We started cultures with 1 million cells from liquid nitrogen. These are quickly thawed and then carefully added to a 15mL tube with 9mL of media. Cells are then spun at 300g for 3 minutes then resuspended and plated in a T-75 flask with 15mL of fresh media. To subculture these cells we collected and spun down the media in the flask at 300g for 5 minutes while trypsinizing the cells in the flask with 5mL of 0.25X trypsin EDTA for 5 min at 37C. After trypsinization 5mL of fresh media is added to the flask and then all the media is collected in a 15mL tube and spun down at 300g for 5 min. Media from the spins is aspirated from the tube without disturbing the pellet. The cells are then resuspended in fresh media and combined before counting. Cells are then counted by acridine orange staining, and 100,000 cells are then added to 15mL of media in a T-75 flask.

2. *Construction of the PLP*: The desired plasmid (pNu3_E) is transformed into BL21(DE3) component cells, and following with DNase I (0.04mg/mL) and lysozyme (0.4mg/mL) to increase the protein extraction efficacy from cell lysates. By applying 10%-40% sucrose gradient untracentrifugation, the PLPs are collected at the third band under bright light, and buffer-exchanged to PLP wash buffer (20mM Tris (pH 8.0 @ 4°C), 15mM MgCl₂, 1mM EDTA, 7mM βME) by 100kDa Amicon centrifugal filter units (100kDa), and dialyzed with PLP wash buffer overnight. Proteins will be fractionated by cation exchange chromatography with 55mL diethylaminoethyl column and eluted by PLP elution buffer (PLP wash buffer + 1M NaCl) with 10-column volume linear gradient. The confirmation of the fractions is analyzed by SDS-PAGE and Agarose gel electrophoresis. PLP-containing fractions are pooled and dialyzed with PLP storage buffer (50mM HEPES (pH7.4 at room temperature), 100mM NaCl, 10mM MgCl₂), and the PLP particles are stored in 4°C until future use.

3. *Conjugation and purification of gpD::Cetuximab*: Reducing gpD (S42C) by incubating with 5 fold TCEP for 1hr at room temperature in maleimide conjugation buffer (50mM HEPES, 250mM NaCl, 5mM EDTA, 5% glycerol at pH 6.6). We then buffer exchanged the antibody into succinimide conjugation buffer (10mM Na₂HPO₄, 1mM KH₂PO₄, 137mM NaCl, 2.7mM KCl, 1mM EDTA, pH 7.2) with 30KDa spin filters. The antibody is then incubated with 5 fold excess of the PEG crosslinker reagent for 30 minutes at room temperature. We then pass the antibody reaction through two zeba spin desalting columns to remove any excess crosslinker reagent before

adding to the reduced gpD for 1 hour at room temperature. We then used a Superose 6 10/300 size exclusion column for purification.

4. *Conjugation and purification of gpD::F5M (fluorescent-5-Maleimide)*: Reducing gpD (S42C) by incubating with 5 fold TCEP for 1hour at room temperature in maleimide conjugation buffer (50mM HEPES (pH 6.6), 250mM NaCl, 5mM EDTA, 5% glycerol at pH 6.6) and fill up the final volume to 1mL with 50mM HEPES (pH 6.6). Fluorescence-5-maleimide is conjugated to the reduced gpD with a concentration of 350 μ M and following with 5mM of EDTA. The conjugation is stored in 4°C overnight, and purified by Zeba™ Spin Desalting Columns twice. The purified conjugation can be stored in 4°C until future use.

5. *Conjugation and purification of gpD::ISD (interferon stimulatory DNA)*: The double stranded DNA is constructed by adding one to one ratio of the leading strand and lagging strand together, and the mixture of the leading strand and the lagging strand is incubated at 95°C for 2 minutes and cooled down to room temperature. Reducing gpD (S42C) by incubating with 5 fold TCEP for 1hr at room temperature in maleimide conjugation buffer (50mM HEPES, 250mM NaCl, 5mM EDTA, 5% glycerol at pH 6.6). DBCO_(PEG)24-4-Maleimide linker is conjugated in 5 fold volume to the reduced gpD, with 1mM of EDTA, and incubated at 37°C for 2 hours. The conjugation is purified by Zeba™ Spin Desalting Columns twice, and buffer-exchanged to annealing buffer (10mM Tris, 50mM NaCl, 1mM EDTA). The double stranded DNA is incubated with 5 fold volume of the linker conjugation at room temperature for 1 hour, and fractionated by anion exchange chromatography with three 5mL HiTrap Q HP columns connected in tandem. The ISD-conjugation fractions are collected and stored in 4°C until future use.

6. *gpD-2'3'cGAMP conjugation*: Reducing gpD (S42C) by incubating with 5-fold TCEP for 1hr at room temperature in maleimide conjugation buffer (50mM HEPES, 250mM NaCl, 5mM EDTA, 5% glycerol at pH 6.6). We then add 10 fold succinimide conjugation buffer (10mM Na₂HPO₄, 1mM KH₂PO₄, 137mM NaCl, 2.7mM KCl, 1mM EDTA, pH 7.2) to the 2'3'cGAMP dissolved in water. The cGAMP is then incubated with 5-fold excess of the PEG crosslinker reagent for 30 minutes at room temperature. Then the reduced gpD and cGAMP incubated with PEG crosslinker are added together and incubate for 1hr at room temperature. Then the reaction mixture is passed through two zeba spin desalting columns to remove any excess PEG crosslinker or cGAMP in the reaction mixture. Reaction is then purified by 3 hitrap 5mL Q columns in tandem with a 0 to 1M NaCl gradient.

7. *PLP Expansion and decoration*: To expand phage like particles we first buffer exchanged them into (2.5M urea, 10mM HEPES) and incubate for 1hr on ice. We then added a 504 fold excess of gpD (wild type and conjugations) in the desired ratio depending on desired final percentages of different components. The gpD conjugations are added individually with the largest being added first and incubated with the particles for 20 minutes. At the end the particles are incubated for 1hr and then stored at 4°C.

8. *Confocal Microscopy*: For confocal microscopy cells were plated on coverslips in 6 well plates one day before fixing with a goal confluency of 50-70%. Cells were then treated with PLP and fixed at time points 0, 30min and 3hr. Following treatment media is removed from the wells and slides are washed with cold PBS. After washing PBS is aspirated and cells are fixed with 1mL of

3.7-4.0% paraformaldehyde in PBS and rocked for 12min. After fixing, cells are washed with PBS before they are permeabilized by the addition of 1mL of 0.1% v/v triton X-100 in cold PBS rocking for 12 minutes. Cells are then washed and 1mL of 1ug/uL DAPI and phalloidin in cold PBS are added to the cells and rocked in the dark for 1 hour. After washing the coverslips are ready to be added to the slide with a 1:4 mixture of diamond anti-fade and supermount mixture. Cells are then imaged by confocal microscopy with the 10X air objective and images are analyzed for integrated density in the FITC channel to determine relative uptake of the particles.

8. *IFN- β ELISA*: For ELISA experiments we treated cells with different concentrations of the secondary messenger cGAMP and particles decorated with Cetuximab and cGAMP. 500,000 cells were plated the day before treatment and were treated in serum free media for 3 hours. After the 3-hour treatment time serum supplemented media was added into each well for the remaining 24 hour period. After 24 hours media from each well was collected and centrifuged at 600g for 5 minutes. After centrifugation supernatant was collected from each sample and then tested using standard ELISA protocol.

4. Stated Goals Not Met: We did not fully meet several of our stated goals for this reporting period. This primarily included assessing STING pathway activation by PLPs in bladder cancer cells. While we observed robust cellular uptake of the PLPs in bladder cancer cells, we have not observed significant STING activation as expected. We repeated experiments in a macrophage cell model and did find STING activation. We suspect there is a threshold of uptake required for STING activation and have been working to modify this by changing the percentage of the targeting antibody (Cetuximab). We also ran into some technical issues with decorating the PLPs with the interferon stimulatory DNA and switched to decorating with cGAMP (another agonist of the STING pathway). This required additional characterization and took some time to complete. We have requested a no-cost extension on this project to complete these tasks as well as the animal studies that were proposed to be completed in late 2022.

Other Achievements:

Nothing to report.

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Two Master's students were trained under this project. The first student, Robert Canfield, is finished the Master's program in Immunology/Microbiology at the Anschutz Campus and has been trained in synthesis and characterization of the PLPs. He was recently accepted into the Pharmaceutical Sciences PhD program and will remain working on this project for his PhD thesis. His major focus in the coming year will be to examine activation of bladder cancer cells by PLPs and will be trained in cell culture, cytokine measurements and Western blotting. In addition, he will begin the animal studies proposed and be trained in animal handling, intravesical delivery and histology. The second student, Yanhao Jiang, graduated from the Master's in Pharmaceutical Sciences program in the School of Pharmacy and was trained in the synthesis and characterization of the PLPs. Yanhao recently joined a PhD program in pharmaceutical sciences at the University of Arizona.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Year 2 plans include the following:

- 1) Complete studies on STING activation in bladder cancer cells (MB49, HTB9 and macrophage cell models)
 - a. Measurement of interferon alpha and beta
 - b. STING pathway activation by Western blotting
- 2) Complete animal studies in an orthotopic model of bladder cancer
 - a. Animal studies will be based on optimized PLPs as determined from the in vitro activation studies

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We ran into some issues with conjugating the interferon stimulatory DNA on the PLPs and made the switch to using cGAMP as a STING agonist which we have had more success in conjugation. We have also run into some issues with activation of the bladder cancer cells by the PLPs, so we have also been examining macrophages which can produce significant amounts of type 1 interferons. We are also examining different bladder cancer cell lines. Our initial studies were with murine cell lines and we have begun experiments with human bladder cancer cells to determine if they have improved IFN- β production. Lastly, we are adjusting the amount of targeting antibody on the PLPs to improve uptake in the bladder cancer cells which should improve immune activation.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report (no human subjects).

Significant changes in use or care of vertebrate animals

Nothing to report (animal studies have not yet been started).

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”*

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal;*

volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report.

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- data or databases;
- physical collections;
- audio or video products;
- software;
- models;
- educational aids or curricula;
- instruments or equipment;
- research material (e.g., Germplasm; cell lines, DNA probes, animal models);
- clinical interventions;
- new business creation; and
- other.

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Name: Jared Brown
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.2

Contribution to Project: Oversaw entire project.
Funding Support: This grant CA190078

Name: Carlos Catalano
Project Role: Co-Investigator
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 1.2

Contribution to Project: Oversaw PLP synthesis.
Funding Support: This grant CA190078

Name: Robert Canfield
Project Role: Master's student
Researcher Identifier (e.g. ORCID ID): N/A

Nearest person month worked: 6

Contribution to Project: Synthesized PLPs and performed cellular uptake studies.

Funding Support: Immunology/Microbiology Master's program at CU Anschutz from Oct 2021 – July 2022. From July 2022 to present Robert has been supported by this grant CA190078.

Name: Yanhao Jiang

Project Role: Master's student

Researcher Identifier (e.g. ORCID ID): N/A

Nearest person month worked: 6

Contribution to Project: Synthesized PLPs and performed cellular uptake studies.

Funding Support: Pharmaceutical Sciences Master's program at CU Anschutz

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.*

- 9. APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*